

Claims

1. A method for testing *Streptococcus pneumoniae* for resistance to penicillin, the method comprising the steps of:

- a) isolating DNA from *Streptococcus pneumoniae*,
- b) hybridizing the DNA obtained in step (a) with at least one sensitivity-specific DNA probe and at least one resistance-specific DNA probe, and
- c) determining whether or not said *Streptococcus pneumoniae* is sensitive to penicillin or not by detecting which probe or probes hybridize.

2. The method according to claim 1, wherein the at least one sensitivity-specific probe is selected from the group of sequences consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and sequences which differ from said sequences by one to four nucleotides, wherein SEQ ID NOS.: 1-13 are, respectively:

AGT CAG CAA CGG GTA AG,
AAC GAA CGA TGG ACG GT,
CAT TTC CAG NCC CCT CCA,
TGC AGA TGC CAC GAT TC,
CTG GTC AGC TTC CTG CG,
TGG TTA TCT AGT CGG GTT AA,
CTG TAT CGA TGA GTC CG,
AAC AGT TCT GCT GAA GAA G,
TAG GAG CAC GCC ATC AGT,
GAC GAA ATG CCT ATC TTG,
CTC TCA ATT TGT AGC ACC T,
CTA TTC TAA CCG TCT GAC A, and

ATC AAA TAC CTA TAT GGT CC;

wherein N is any nucleotide.

3. The method according to claim 2, wherein the at least one sensitivity-specific probe is selected from the group of sequences consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13.

4. The method according to claim 1, wherein the at least one resistance-specific probe is selected from the group of sequences consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 and sequences which differ from said sequences by one to four nucleotides, wherein SEQ ID NOS.: 14-19 are, respectively:

TGG AGA ATA NTT CAA TAG N,
GTC TAC TTG AAC AAA AAA TG,
TTA GTT GGG ACG GAC CCT,
GTA ACN NTT CAA CAG CCT,
CTC CGA NCA ATA CGT CTC T, and
GCT CCA GAT NAA ATG TTT GT;
wherein N is any nucleotide.

5. The method according to claim 4, wherein the at least one resistance-specific probe is selected from the group of sequences consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19.

6. The method according to claim 1, wherein the probes are labeled radioactively.

7. The method according to claim 1, wherein the conditions for hybridization are stringent conditions.

8. The method according to claim 1, wherein DNA obtained in step (a) is hybridized with more than one resistance-specific DNA probe and more than one sensitivity-specific DNA probe.

9. The method according to claim 2, wherein DNA obtained in step (a) is hybridized with more than one sensitivity-specific DNA probe chosen from among said group.

10. The method according to claim 4, wherein DNA obtained in step (a) is hybridized with more than one resistance-specific DNA probe chosen from among said group.

11. A method for testing *Streptococcus pneumoniae* for resistance to penicillin, the method comprising the steps of:

- a) isolating DNA from *Streptococcus pneumoniae*,
- b) exposing the DNA obtained in step (a) with at least one sensitivity-specific DNA probe and at least one resistance-specific DNA probe under conditions which can permit hybridization, and
- c) determining whether or not said *Streptococcus pneumoniae* strain is sensitive to penicillin or not by detecting which probe or probes hybridize;

wherein the at least one sensitivity-specific probe is selected from the group of sequences consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and sequences which differ from these sequences by one to four nucleotides, wherein SEQ ID NOS.: 1-13 are, respectively:

AGT CAG CAA CGG GTA AG,
AAC GAA CGA TGG ACG GT,
CAT TTC CAG NCC CCT CCA,
TGC AGA TGC CAC GAT TC,
CTG GTC AGC TTC CTG CG,
TGG TTA TCT AGT CGG GTT AA,
CTG TAT CGA TGA GTC CG,
AAC AGT TCT GCT GAA GAA G,
TAG GAG CAC GCC ATC AGT,
GAC GAA ATG CCT ATC TTG,
CTC TCA ATT TGT AGC ACC T,
CTA TTC TAA CCG TCT GAC A, and
ATC AAA TAC CTA TAT GGT CC;

wherein N is any nucleotide; and wherein the at least one resistance-specific probe is selected from the group of sequences consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 and sequences which differ from these sequences by one to four nucleotides, wherein SEQ ID NOS.: 14-19 are, respectively:

TGG AGA ATA NTT CAA TAG N,
GTC TAC TTG AAC AAA AAA TG,
TTA GTT GGG ACG GAC CCT,
GTA ACN NTT CAA CAG CCT,
CTC CGA NCA ATA CGT CTC T, and
GCT CCA GAT NAA ATG TTT GT;

wherein N is any nucleotide.

12. The method of claim 11, wherein the at least one sensitivity-specific probe is selected from the group of sequences consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ

ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13; and,

wherein the at least one resistance-specific probe is probes which specifically hybridize to the DNA of antibiotic resistant strains and are selected from the group of sequences consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19.

13. The method of claim 11, wherein the DNA isolated in step (a) is obtained from a strain of bacteria having unknown antibiotic sensitivity or resistance.

14. The method of claim 11, wherein DNA from said *Streptococcus pneumonia* is exposed to more than one different sensitivity-specific DNA probe selected from the group of sequences consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and sequences which differ from these sequences by one to four nucleotides, under conditions which can permit hybridization; and,

wherein DNA from said *Streptococcus pneumonia* is exposed to more than one different resistance-specific DNA probe selected from the group of sequences consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 and sequences which differ from these sequences by one to four nucleotides, under conditions which can permit hybridization.

15. A penicillin sensitivity-specific DNA probe for determining penicillin sensitivity in *Streptococcus pneumonia* selected from the group of sequences consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID

NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and sequences which differ from said sequences by one to four nucleotides, wherein SEQ ID NOS.: 1-13 are, respectively:

AGT CAG CAA CGG GTA AG,
AAC GAA CGA TGG ACG GT,
CAT TTC CAG NCC CCT CCA,
TGC AGA TGC CAC GAT TC,
CTG GTC AGC TTC CTG CG,
TGG TTA TCT AGT CGG GTT AA,
CTG TAT CGA TGA GTC CG,
AAC AGT TCT GCT GAA GAA G,
TAG GAG CAC GCC ATC AGT,
GAC GAA ATG CCT ATC TTG,
CTC TCA ATT TGT AGC ACC T,
CTA TTC TAA CCG TCT GAC A, and
ATC AAA TAC CTA TAT GGT CC;
wherein N is any nucleotide.

16. A penicillin resistance-specific DNA probe for determining penicillin resistance in *Streptococcus pneumonia* selected from the group of sequences consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 and sequences which differ from said sequences by one to four nucleotides, wherein SEQ ID NOS.: 14-19 are, respectively:

TGG AGA ATA NTT CAA TAG N,
GTC TAC TTG AAC AAA AAA TG,
TTA GTT GGG ACG GAC CCT,
GTA ACN NTT CAA CAG CCT,
CTC CGA NCA ATA CGT CTC T, and

GCT CCA GAT NAA ATG TTT GT;

wherein N is any nucleotide.

17. A kit for performing the method of claim 1, comprising penicillin sensitivity-specific DNA probes for determining penicillin sensitivity in *Streptococcus pneumonia* selected from the group of sequences consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and sequences which differ from said sequences by one to four nucleotides, wherein SEQ ID NOS.: 1-13 are, respectively:

AGT CAG CAA CGG GTA AG,
AAC GAA CGA TGG ACG GT,
CAT TTC CAG NCC CCT CCA,
TGC AGA TGC CAC GAT TC,
CTG GTC AGC TTC CTG CG,
TGG TTA TCT AGT CGG GTT AA,
CTG TAT CGA TGA GTC CG,
AAC AGT TCT GCT GAA GAA G,
TAG GAG CAC GCC ATC AGT,
GAC GAA ATG CCT ATC TTG,
CTC TCA ATT TGT AGC ACC T,
CTA TTC TAA CCG TCT GAC A, and
ATC AAA TAC CTA TAT GGT CC;

wherein N is any nucleotide;

and further comprising penicillin resistance-specific DNA probes for determining penicillin resistance in *Streptococcus pneumonia* selected from the group of sequences consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 and sequences which differ from

said sequences by one to four nucleotides, wherein SEQ ID NOS.: 14-19 are, respectively:

TGG AGA ATA NTT CAA TAG N,
GTC TAC TTG AAC AAA AAA TG,
TTA GTT GGG ACG GAC CCT,
GTA ACN NTT CAA CAG CCT,
CTC CGA NCA ATA CGT CTC T, and
GCT CCA GAT NAA ATG TTT GT;
wherein N is any nucleotide.